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COMPARATIVE STUDY OF ENZYMATIC TRANSESTERIFICATION OF *Jatropha* OIL USING LIPASE FROM *Jatropha curcas* and *Jatropha gossipyfolia*

[1]Gayatri Nahak, [1]Debyani Samantray, [2]N.K. Mohapatra and [1]R.K. Sahu
[1]B.J.B. Autonomous College, Bhubaneswar, Orissa, [2]Academy Management and Information Technology, Bhubaneswar, Orissa

ABSTRACT
Biodiesel consists of monoalkyl esters of long chain fatty acids. It is produced from vegetable oils or fats either by chemical transesterification with methanol or ethanol. The cost of lipases and the relatively slower reaction rate remain as the major obstacles for enzymatic production of biodiesel as opposed to the conventional chemical processes. The enzymatic process offers several advantages over the chemical routes. The handicap of increase in process cost because of the enzyme can be overcome by using efficient production process for enzyme and using reusable derivative of enzymes, such as immobilized enzyme. Numerous strategies available in the area of non-aqueous enzymology can be exploited during the enzymatic alcoholysis for the biodiesel production. The paper reviews the starting oils usually employed in biodiesel production, the process for transforming them to biodiesel playing particular emphasis on enzymatic transesterification the sources of production and characterization of vegetable oils and their methyl ester as the substitute of the petroleum fuel.

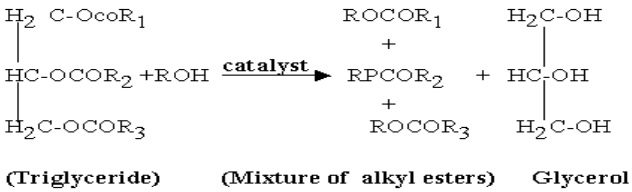
KEYWORDS: Biodiesel, Enzymatic transesterification, *Jatropha* oil, Lipase, *J. curcas, J. gossipyfolia*.

INTRODUCTION

Many researchers have concluded that vegetable oils hold promises as alternative fuels for diesel engines (Goering *et al*., 1982; Bagby *et al*; 1987). However, using raw vegetable oils for diesel engines can cause numerous engine related problems (Korus *et al.*, 1982; Vander Walt and Hugo,1982). The increased viscosity and low volatility of vegetable oils lead to severe engine deposits, injector coking and piston ring sticking (Vellguth, 1983; Clark *et al.*, 1984; Pestes and Stanislao,1984; Perkins and Peterson,1991). However these effects can be reduced or eliminated through transesterification of vegetable oil to methyl esters, commonly known as Biodiesel (Zhang e*t al.*, 1988;Perkins and Peterson.,1991). Consequently, considerable effort has gone into developing vegetable oil derivatives that approximate that properties and performance of hydrocarbon-based diesel fuels problems encountered in substituting triglycerides for diesel fuels are mostly associated with their high viscosity, low volatility, and polyunsaturated character (Srivastava *et al*.,2000). As an alternative to diesel fuel, Biodiesel must be technically feasible, economically competitive, environmentally acceptable and readily available. Now-a-days Biodiesel fuel is used in public traffic for performing from engines, lighting and heating of rooms in specific condition (Haas, 2005; Schlautman *et al*., 1986; Tomasevic and Siler Marinkove,2003). Yamane *et al*,2001 recently reported that a biodiesel fuel with good ignitability, such as one with a high methyle oleate content, gives lower levels of NO, Hydrocarbons, HCHO, CH_3HO and HCOOH. Since Biodiese is an Oxygenated fuel having an O_2 mass fraction of10%. In addition, Sheehan *et al*, 1998 carried out life cycle analysis and found that the benefit of using Biodiesel is proportionate to the level of blending with petroleum diesel. Three main processes have been investigated in attempts to overcome these drawbacks and allow vegetable oil and waste to be utilized as a viable alternative fuel: Pyrolysis, Micro-emulsification, and Transesterification. Transestrification provides a fuel viscosity that is close to that of No.2 diesel fuel.

Transesterification is also called as alcoholysis, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis, except that an alcohol is employed instead of water (Meher *et al.*, 2006; Srivastava and Prasad. 2000). Suitable alcohols include methanol, ethanol, propanol, butanol, and amyl alcohol. Methanol and ethanol are utilized most frequently, especially methanol because of its low

cost and its physical and chemical advantages. This process has been widely used to reduce the viscosity of triglycerides, thereby enhancing the physical properties of renewable fuels to improve engine performance (Clark *et al.,* 1984). Transesterification of triglycerides produces fatty acid alkyl esters and glycerol. The glycerol layer settles down at the bottom of the reaction vessel. Diglycerides and monoglycerides are the intermediates in this process. The mechanism of transesterification is described as follows.
Figure-1(a)



The transesterification reaction with alcohol represented by the general equation shown in Fig-1(a). The first step is the conservation of triglycerides to diglycerides which is followed by the conversion of diglycerides to monoglycerides and of monoglycerides to glycerol, yielding one methyle ester molecule from each glycerides at each step (Freedman *et al*.,1986 and Noureddimi *et al*.,1997)

Although chemical transesterification using an alkali-catalyst process gives high conversion levels of triglycerides to their corresponding methyl esters in short reaction times, the reaction has several drawbacks: it is energy intensive, recovery of glycerol is difficult, the acidic or alkaline catalyst has to be removed from the product, alkaline waste –water requires treatment and free fatty acids and water interfere with the reaction. To overcome such problems associated with chemical catalyst for production of Biodiesel, enzymatic transesterification process using lipase have been developed. The enzymatic process offers several advantages over the chemical routes. The handicap of increase in process cost because of the enzyme can be overcome by using efficient production process for enzyme and using reusable derivative of enzymes, such as immobilized enzyme. Numerous strategies available in the area of non-aqueous enzymology can be exploited during the enzymatic alcoholysis for the biodiesel production. The paper reviews the starting oils usually employed in biodiesel production, the process for transforming them to biodiesel playing particular emphasis on enzymatic transesterification the sources of production and characterization of vegetable oils and their methyl ester as the substitute of the petroleum fuel.

The interest in the application of enzymes to organic synthesis has been growing rapidly in recent years. A lot of attention has been devoted to attempts at utilizing the catalytic properties of lipase in organic synthesis. The catalytic activity and selectivity of enzymes depend on, among other things, the structure of the reacting substances, the process conditions, the kinds of solvents, and the presence of water (Gryglewicz *et al*.,2000). Lipase (triglycerol acylhydrolase, E.C.3.1.1.3) are enzymes widely distributed among animals, plants, and micro-organisms that catalyze the reversible hydrolysis of glycerol ester bond and therefore, also the synthesis of glycerol ester.

In nature, lipase used only for hydrolysis. Under certain circumstances, lipases also catalyze a transeterification reaction. Lipase can be used in low-water environment as excellent tool for the transestrification of commercial triglycerides, and/or their derivatives, to synthesize a growing range of products of potential industrial interest (Pirozzi., 2003).the industrial applications of lipases have grown rapidly in recent years are likely to markedly expand further in the coming year. Lipase may be used to produce fatty acids (Linder *et al*.,1993), biosurfactants (Edmundo *et al.,* 1998), aroma and flavor compounds (Athawale *et al*.,2003), lubricant and solvent esters (Hills, 2003), amides and thiol esters (Gandhi 1997). There have been a number of studies, which reported lipase catalyzed transesterification with and without organic solvents. For diesel fuel, ethyl ester is preferred because ethanol can be produced from biomass and is less toxic, but conventional alcoholysis with ethanol gives low yield.
The lipase catalyzed reaction can be classified as follows.

I Hydrolysis:

$$R^1COOR^2+H_2O \rightarrow R^1COOH+R^2OH$$

II. Synthesis: reaction under this category can be further divided.

1. Esterfication

$$R^1COOH+R^2OH \rightarrow R^1COOR^2+H_2O$$

2. Transesterfication

Alcoholysis

$$R^1COO\ R^2+R^3OH \rightarrow R^1COO\ R^3+R^2OH$$

Acidolysis

$$R^1COO\ R^2+R^3COOH \rightarrow R^3COO\ R^2 + R^1COOH$$

Most interesting is the utilization of lipase for catalyzing the synthesis of simple ester of vegetable oil or other agricultured lipid feedstock e.g. the lipase catalyzed alcoholysis of sunflower oil under anhydrous conditions (Mittelbach, 1990). Dosssat *et al*., 1999 reported transesterification of high oleic sunflower oil with butanol by the immobilized lipozyme R in n-haxane.the reaction was carried out in a continuous packed bed reactor. Without an additional organic solvent, Linko *et al*.,1994 studied lipase catalyzed transestrification of low erucic acid rapeseed oil and 2-ethyl-1-hexanol. The optimal transestrification condition was an oil/alcohol/molar ratio 1:2:8, a minimum of 1.0 %(W/W) added water and with a temperature of 37^0C-55^0C. Under the optimal condition, a nearly complete conversion was obtained in one hour with 14.6 %(W/W) lipase, whereas 0.3 %(W/W) lipase required 10hrs.for similar results. However, at 60^0C lipase was clearly inactivated under the experimental condition.

Abigor *et al*., 2000 reported lipase-catalysed production of alkyl ester by transesterification of palm kernel and coconut oil with different alcohols using PS30 (*Pseudomonas cepacia*) lipase as a catalyst. In the conversion of palm kernel oil to alkyl esters, without any added solvent to the reaction mixture the highest conversion was given by ethanol(72%), followed by tert-butanol(62%), butanol(42%), propanol(42%), and isopropanol(24%), while only 15% methyl esters was observed with methanol. Through 3-step addition of methanol, Du *et al.,* 2003 amined lipase-catalyzed transeterification of Soya oil in continuous batch operation. They found that in non-continuous batch operation, the optimal oil/alcohol ratio and temperature were 1:4 and 40^0-50^0C, because either at higher (1:5) or lower (1:3) methanol concentration would decrease the methyl esters yield to some degree. In this condition, methyl esters yield reach up 92% after 6hrs.reaction. However, during the continuous batch operation lipase lost its activity dramatically when the methanol/oil/molar ratio was 2:1.the optimal molar ratio of oil (alcohol and temperature) were 1:1 and 30^0C (Du *et al*., 2003). More details about lipase-catalyzed and enzymatic transestrification of *Jatropha* oil with methyl and ethyl alcohol will be presented in this paper.

MATERIALS AND METHODS

Plant Materials

The seeds and leaves of *Jatropha curcas* and *Jatropha gossipyfolia* were brought from Department of Forestry, OUAT, Bhubaneswar, and Orissa. The seeds were deshelled manually and mechanically pressed and separated from impurity with the help of separating funnel. Then oil was used for physio-chemical characterization, enzymatic transesterification.

Chemical and Reagents
Petroleum ether (40-60^{0}C), 1% phenolphthalein, 95% ethanol, 0.1N potassium hydroxide,0.5N HCl, KOH, Silica gel, $CaCl_2$, Benzene, Phosphate buffer(pH 7.3)50mM, Sodium taurocholate(Bile salt), Acetone, Ammonium sulphate, 50% H_2SO_4 and double distilled water.

Instrumentations
Soxhlet apparatus, Rotary shaker, Cooling Centrifuge, and TLC apparatus and mortar and pestle.

Oil Extraction
For the extraction of *Jatropha* two main methods have been identified. 1. Mechanical oil extraction and 2. Chemical oil extraction (Aderibigbe *et al*,1997 and Forson *et al*, 2004).

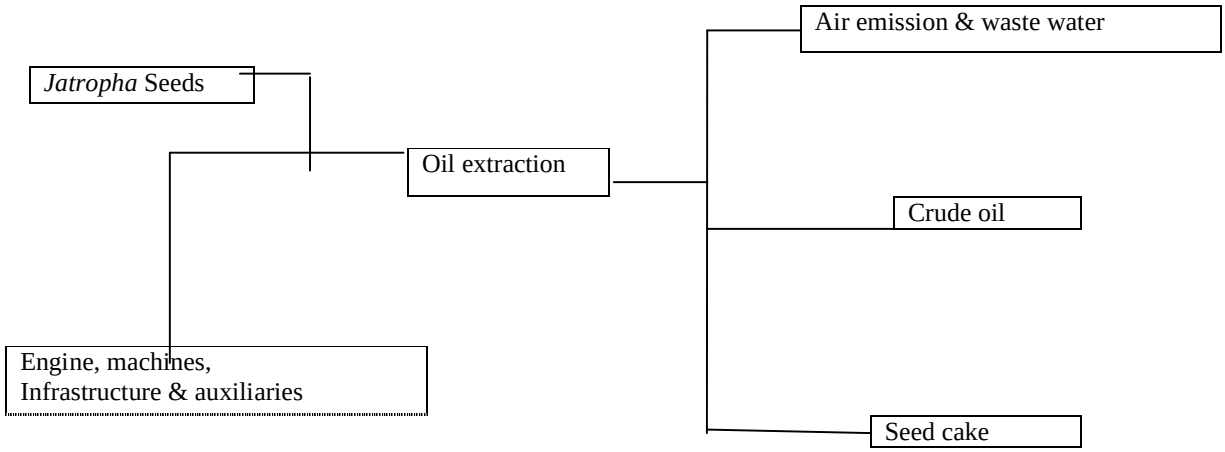


Figure-1(b): (Flow chart of Oil extraction Unit process)

Physio-chemical Estimation

All the physiochemical tests were done by the method followed by Sadasivam and Manickam., 2008.

Estimation of oil content
A piece of filter paper was folded in such a way to hold the seed meal and then second filter paper was wrapped which was left open at the top like a thimble. Then a piece of cotton wool was placed at the top for evenly distribution of solvent as it drops on the sample during extraction. Then a sample packet was placed in the extractor of soxhlet apparatus and oil was extracted with petroleum ether for 6h without interruption by gentle heating. Then it was allowed to cool and dismantle the extraction flask. Then ether was evaporated on a steam or water bath until no odour of ether remains and cooled to room temperature.

Calculation:

$$\text{Oil in ground sample (\%)} = \frac{Wt\ of\ Oil\ (g)}{} \times 100$$

$$\text{Oil in dry wt. Basis (\%)} = \frac{Oil in\ ground\ \ sample}{Moisture\ \ in\ whole\ \ seed} \times 100$$

Estimation of acid value
5 gm oil was dissolved in 50ml of neutral solvent in 250ml conical flask and few drops of phenolphthalein were added. Then it was titrated against 0.1N KOH and shaken constantly until a pink color persists for 15 seconds is obtained.

Calculation

Acid value (mgKOH/gm) = $\frac{Titrevalue \times Normality\ of\ KOH \times 56.1}{Wt\ of\ Sample\ (g)}$

Estimation of Saponification Value

5gm of oil was taken into flask and 50ml. of an alcoholic KOH was added from a burette by allowing it to drain for a period of time. Simultaneously a blank was conducted by taking only 50ml. alcoholic KOH without oil. Air condenser was connected to flask and boiled gentle for about one hour. After flask and condenser get cooled, inside of the condenser was rinsed down with a little distilled water. Then 1ml. of indicator was added and titrated against 0.5N HCl until pink color just disappeared.

Calculation

Saponification value =

$$\frac{Titre\ value\ of\ blank - Titre\ value\ of\ samples \times 28.05}{Wt\ of\ Sample\ (gm)}$$

Biodiesel production

This includes extraction and purification of lipase and its transesterification.

Free enzyme Preparation

The seeds of *J. curcas* and *J. gossipyfolia* were taken in Petri dishes and kept for germination. The germinated seeds were homogenized in mortar and pastel with cold petroleum ether. Similarly the fresh leaves of *J. curcas* and *J.gossipyfolia* were taken and washed properly with distilled water and were homogenized in mortar pestle with cold petroleum ether. Then it was centrifuged successively with ether mixture and then finally ground with cold acetone to fine powder, air dried and preserved at -4^0C. The enzyme was extracted from acetone powder by phosphate buffer (pH7.3). Then they were centrifuged at 15000g for 10min at -4^0C. The supernatant was preserved for further analysis ad the residue was discarded.

The crude enzyme extract was partially purified by salting out method using 70% ammonium sulfate. The precipitated proteins from all samples were recovered after discarding the supernatant. The precipitate was redissolved in phosphate buffer (50mM) pH 7.3 and dialyzed to make it free from ammonium sulfate. The dialyzed samples were taken as enzyme source.

Assay of lipase from acetone powder

The prepared acetone powder (2gm.) was slightly ground with mortar and pestle with buffer solution (30% KOH+ 50% KH_2PO_4+ 20ml ice cold H_2O). Then it was centrifuged at 15000 rpm for 15 min. The pellet was discarded and the supernatant was used as enzyme source (Sadasivam and Manickam., 2008).

To 20ml. of substrate 5ml. of phosphate buffer was added and the contents were stirred slowly by keeping the beaker on top of a magnetic stirrer-hot plate maintaining the temperature at 35^{o}C and pH 7.0. 0.5ml. of enzyme was added and the pH was recorded immediately at zero time with timer on (pH at zero time). At regular intervals (10 min) or as the pH drops by about 0.2 units 0.1N NaOH was added to bring the pH back to the original level. This titration was repeated for 30-60 min and the volume of NaOH consumed was noted to estimate the protein content in the enzyme sample.

Calculation

The enzyme activity as the amount of enzyme required to release one milliequivalent of free fatty acid/ min./gm. sample and specific activity as milliequivalent/ min./ mg. protein.

$$\text{Enz. Activity(meq./min./gm. Sample)} = \frac{\text{Vol. of the alkali consumed} \times \text{Normality of alkali}}{\text{Wt. of the sample} \times \text{Time(min)}}$$

Enzymatic Transesterification

The enzymatic transesterification was done followed by the method *Jatropha* oil (10gm) and ethanol (2gm) were taken in the ratio of 1:4 (mole mle^{-1}) in a 100 ml conical flask. To this mixture different concentrations of enzymes showing different activity of enzymes in terms of milliequivalent/min/mg protein were taken in solution form were added and stirred. Then heated to 40^0C with constant shaking at 200 rpm. Then the samples were withdrawn and analyzed for maximum conversion.

Test for complete esterification

To 100ml. conical flask containing 2gm. of esters, 5gms.mixture of silica-G: anhydrous $CaCl_2$ (W/W) in the ratio 1:1 was added. It was mixed thoroughly with a glass rod. Then 10ml. of benzene was added and was shaken for ½ to 1min centrifugation was done for short period. The residue was washed with benzene and then it was evaporated the solvent mixture to its original volume of ester oil.

TLC for ethyl ester

Purification of ester mixture was tested by TLC. A chromatographic plate was prepared by using 20×20m.glass plate over layered with silica gel-G slurry of 2mm. thickness and having a pore size of 250m. The set specification was obtained by making the slurry in water in the production 1:2. The slurry was applied by spreader after adjusting the thickness to 2mm. The plates were dried at room temperature and activated before use 120C for 1hr.

The ester mixtures were applied at different spots with the unesterified oil at one end as control. At the other end commercial diesel was also applied for comparative study. The applied sample was dried in air and put inside the chromatographic tank containing solvent mixture of petroleum ether and acetic acid in the proportion 80:20:1. A chromatographic running is allowed till the solvent front reach few centimeters below the top. The plates were taken out and dried in air and was spread with the developer (50% H_2SO_4) and was put inside the hot air oven for 1-2 hr. The absence of spots in case of ester mixture against spots developed by the control confirms the complete esterification.

RESULTS AND DISCUSSION

The physiochemical properties were assessed in the P.G. Department of Biotechnology, Biochemistry laboratory and results are presented in Table-1. In vitro studies of transesterification were conducted to draw out an inference of maximum yield of Biodiesel using two different sources for enzyme lipase from *Jatropha curcas* and *Jatropha gossipyfolia* plant parts. Experiments were conducted to study enzymatic transesterification with standardized protocol with respect to reaction environment. The physiological properties of *Jatropha* oil suit to go for Biodiesel production as the oil content contains highly unsaturated long chain fatty acid as the best substitute to any other source materials for *in vitro* studies.

Effect of ethanol and enzyme concentration on Biodiesel yield in *J. curcas* and *J. gossipifolia*

The preliminary studies to determine the optimum quantity of ethanol, catalyst lipase reaction temperature and reaction time required for transesterification of *Jatropha* oil were conducted dry varying concentration of ethanol from 10 to 20% lipase concentration 2.0, 2.5 and 3.0gm.equivalent, reaction temperature (40^0C) and reaction time 8 hour. To 100ml conical flask containing 10gm. *Jatropha* oil, varying concentration of ethanol 1.0, 2.5 and 3.0gm equivalent. Each mixture was stirred and heated to 40^0Cwith constant shaking at 200rpm for 8hr. samples were withdrawn after reaction period and analyzed for maximum conversion. In this Table-2 it shows that the combined treatment (in case of *J. curcas* seed extract) at enzyme 3.0 gm equivalent and 0.2 gm. Ethanol shows higher yield of biodiesel i.e. 8.25gm. In case of leaf extract the combined treatment at 3.0 gm. equivalent and 0.2 gm, ethanol shows higher yield of Biodiesel i.e. 7.10 gm. Where as in case of *J. gossipifolia* it shows little less as comparison to *J. curcas*. In Table-3 it shows that the enzyme concentration 3.0 gm. Equivalent in presence of ethanol 1:4 molar ratio i.e. 2 gm. /10gm. of oil (in case of *J.gossipyfolia*) shows preferably higher yield i.e. 7.85 gm similarly in case of leaf extract the combined value of enzyme at 3.0 gm. equivalent and ethanol at 2.0 gm. gives higher yield i.e. 5.85 gm.

Enzymatic transesterification with specific reference to ester yield by lipase from plant parts of *J.curcas* and *J. gossipyfolia* such as seeds, stems and leaves were studied. The extraction, partial purification and enzyme assay were done as per standard protocol. The yields of Biodiesel were studied with the use of lipase only in free preparation. Ethanol was used at various concentrations ranging from 10-25% keeping other factors constant for suitable environmental condition for enzymatic catalyst on the basis of finding (Shah, *et al*, 2003). As we know lipase possesses unique feature of acting at interface between aqueous and organic phase and its activation involves in making the restriction of active site requiring oil water interface. Therefore studies were made taking water at various levels along with enzyme at various concentrations. The reactions were carried out according to reaction set up and optimization conditions described earlier. The results obtained in summarized and put at a glance in Fig-2 and Fig-3.

Reaction temperature in case of enzyme catalyzed transesterification shows in conformity with other researches and appears to be at optimum 40^0C when reaction time allowed 8hrs. The yield of Biodiesel is visibly significant for all sources of enzyme catalyzed reactions that the yield of Biodiesel remains in maximum range with depression of glycerol output. The level of biodiesel yield goes up to above at optimal reaction environment which may exceed beyond with further intensive investigation (Du *et al.*, 2003). Although reports available shows once preferential approach to go for chemical catalysis with a base material as catalyst taking into reaction timing and cost of study as point of consideration but at the same time the data and results show the importance of biochemical transesterification can't be denied. More over when the removal of glycerol as by-product is considered, biochemical transesterification is no doubt preferred to chemical transesterification. In order to shift the reaction to the right an alcohol excess (molar ratio alcohol:oil = 6:1) and a catalyst (NaOH, KOH at 20% by weight on oil basis) are necessary (Chitra *et al.*, 2005).An optimal ester yield of 98% is achieved after 90 min. of reaction at 60°C (Chitra *et al.*, 2005). Crude glycerol is separated and can be used as a raw material for soap production or other cosmetica.

Enzyme extract obtained from *Jatropha* seed as compared to that of leaf shows better result, which obvious for the reason that lipase from the leaf is being metabolized before translocated to other parts of the plant, where as lipase in the germinated seeds is in activated form for hydrolysis of triglyceride and other lipids to support the carbon source for gluconeogenesis in the energy supplement.

When we look into the efficiency of transesterification of both the species of *Jatropha* plant, *J.curcas* is in preferential position as compared to *J.gossipyfolia* for the reason of compatibility of enzyme with the oil from same source as the substrate i.e. *J.curcas* oil is being taken for study of transestrification.
Accumulation of emulsion when leaf extract is taken as source is more in both the species compared to seed extract most probably due to increased salt concentration in leaf extract. Under circumstances intermittent withdrawal of emulsions with stepwise addition of ethanol would have been improved the situation in increasing the yield.

The comparative figures (Fig-2 and Fig-3) of the Biodiesel yield obtained from these two species of *Jatropha* show the use of lipase from *Jatropha* seed as better performance followed by *Jatropha* leaves in both the cases. Since other variable such as alcohol concentration is constant in all the treatments, the activity index of lipase at 3gm.equivalent appears to have important consideration compared to total protein/enzyme content in transesterification as given in the Table-2(a) and Table-2(b).

Interestingly the enzyme catalyzed transesterification shows compatible results (Table-3 and Table-4) in conversion percentage to the extent of nearly 82-85% with the use of same quantity of alcohol for alcholysis of *Jatropha* oil as is required for chemical process with reduction in temperature requirement and lengthening reaction time. The reports (Vacek *et al.*, 2001) show higher yield to the extent of 98% because commercial enzyme in purified form are being used. The concentration of glycerol and other by products and enzyme catalyzed transesterification plays a vital role as increased level depressed the conversion in the process of catalysis. Transesterification being an equilibrium reaction, shifting towards product formation required use of excess alcohol proportionally. The data and graphical representation

show 20% alcohol concentration with relation to oil/glycerol both in enzyme and base catalyzed transesterification affect yield of Biodiesel.

Since removal of glycerol from the product pool during the progress of product catalysis hasn't been done due to technical constraints, it is worthwhile to mention the yield of Biodiesel may likely to increase further in the system (Belafi-Bako *et al*., 2002). The decline in ester yield beyond use of 20% alcohol may be attributed to non removal of product after the steady state as it is evident from the result that reduction of Biodiesel yield is visibly significant with increase in accumulation of other by-product.

Fig-6 shows TLC results for different oil samples along with esterified and unestrified *Jatropha* oil obtained after incubation for 12hrs. Moreover it is interesting to observe that although the ester mixture confirms the BIS standard, the experimental findings of the chromatogram of the ester mixture clearly indicates variability of ester mixture composition in varying chain length. Therefore in absence of GLC quantification and characterization of ester mixture is not possible in the present investigation. However the fact is that exploitation of the different sources of enzymes as in the study being the cheapest, leave enough scope of further research in the transesterification process to standardize reactant concentrations, reaction timing etc. with modified reactor design for encouraging result in future.

CONCLUSION

Many studies have been done all over the globe after releasing the importance of Biodiesel. Since then workers are in continuous effort to develop a suitable protocol for effective, efficient and economic conversion of non edible vegetable oil to Biodiesel to meet the challenge for the forth coming energy crises. The result obtained are quite encourgable for further investigation because the process of esterification is confined to eco-friendly enzymatic transesterification using natural biocatalyst from the same source with that oil. It can't be concluded with the information available beyond all reasonable doubt that the use of enzyme from *Jatropha curcas* is the best source of catalyst in this process, but the comparative parameter with respect to yield from literature available with that of commercial lipase derived from microbial source is better alternate in quantity terms. However when we consider the cost of production of Biodiesel taking all factors and consideration lower yield of Biodiesel with natural lipase appears to be more profitable proposition. So in the case with species of *J.gossipyfolia* using as the source of enzyme in transesterification too.

Therefore the overall use of different species of *Jatropha* oil isn't worthy and desirable in terms of quantity and quality of the product, which is confirmed from the ester yield with thorough investigation to devise a standard protocol to go for commercial production of Biodiesel. More research is necessary to get a good insight in the environmental sustainability of this production system. The land use impact is an absolute must to address those sustainability issues.

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Table-1: Physio-chemical properties of Biodiesel

Characters	Parameters
Oil content	45%
Acid value	23.2
Saponification value	193.0
Iodine value	93.5
Viscosity	36.5

Table-2(a): Effect of ethanol and enzyme concentration on Biodiesel yield in *J. curcas*

Sources of enzyme	Enzyme conc. (gm.equ.) / **Ethanol Conc. (0gm**	**Biodiesel in gms**		
		2.0	**2.5**	**3.0**
Seed extract	**1.0**	**6.32**	**6.25**	**6.32**
	1.5	**7.51**	**7.63**	**7.44**
	2.0	**8.05**	**7.91**	**8.25**
Leaf extract	**1.0**	**6.30**	**6.44**	**6.50**
	1.5	**6.89**	**6.90**	**6.95**
	2.0	**6.98**	**7.00**	**7.10**

Table-2(b): Effect of ethanol and enzyme concentration on Biodiesel yield in *J. gossipyfolia*

Sources of enzyme	**Enzyme conc. (gm. Equ.)** / **Ethanol Conc. (gm)**	**Biodiesel in gms**		
		2.0	**2.5**	**3.0**
Seed extract	**1.0**	**6.25**	**6.27**	**6.30**
	1.5	**6.55**	**6.78**	**6.98**
	2.0	**7.38**	**6.58**	**7.85**
Leaf extract	**1.0**	**5.12**	**5.35**	**5.48**
	1.5	**5.5**	**5.68**	**5.70**
	2.0	**5.75**	**5.80**	**5.85**

Table – 3 : Average yield in lipase catalysed transesterification of jatropha curcas under different treatments.

No. of treatments	Oil in (gm)	Ethanol in (gm)	Enzyme in (gm equ.)	Biodiesel (gm)	Emulsion (gm)	Loss (gm)	% Conversion
TJC_s-1	10	1.0	2.0	6.32	4.47	0.21	63.2
TJC_s-2	10	1.0	2.5	6.25	4.50	0.25	62.5
TJC_s-3	10	1.0	3.0	6.32	4.46	0.22	63.2
TJC_s-4	10	1.5	2.0	7.51	3.25	0.24	75.1
TJC_s-5	10	1.5	2.5	7.63	3.16	0.21	76.3
TJC_s-6	10	1.5	3.0	7.44	3.31	0.25	74.4
TJC_s-7	10	2.0	2.0	8.05	3.73	0.22	80.5
TJC_s-8	10	2.0	2.5	7.91	3.85	0.24	79.1
TJC_s-9	10	2.0	3.0	8.25	3.50	0.25	82.5
TJC_L-1	10	1.0	2.0	6.30	4.48	0.22	63.0
TJC_L-2	10	1.0	2.5	6.44	4.33	0.23	64.4
TJC_L-3	10	1.0	3.0	6.50	4.28	0.22	65.0
TJC_L-4	10	1.5	2.0	6.89	4.40	0.21	68.9
TJC_L-5	10	1.5	2.5	6.90	4.36	0.24	69.0
TJC_L-6	10	1.5	3.0	6.95	4.23	0.23	69.5
TJC_L-7	10	2.0	2.0	6.98	4.76	0.26	69.8
TJC_L-8	10	2.0	2.5	7.00	4.75	0.25	70.0
TJC_L-9	10	2.0	3.0	7.10	4.67	0.25	71.0

In the Table- 3 : It is apparent at optimum temperature i.e 40^0 c and reaction time i.e 8 hrs . which was standardized by other workers shows the conversion percentage using lipase from *J. curcus* shows higher yield i.e 82.5% in case of seed extract and 71% in leaf extract.

Table – 4: Average yield in lipase catalysed transesterification of *J. gossipyfolia* under different treatments.

No. of treatments	Oil in (gm)	Ethanol in (gm)	Enzyme in (gm equ.)	Biodiesel (gm)	Emulsion (gm)	Loss (gm)	% Conversion
TJC_s-1	10	1.0	2.0	6.25	4.55	0.20	62.5
TJC_s-2	10	1.0	2.5	6.27	4.52	0.21	62.7
TJC_s-3	10	1.0	3.0	6.30	4.45	0.25	63.0
TJC_s-4	10	1.5	2.0	6.55	4.72	0.23	65.5
TJC_s-5	10	1.5	2.5	6.78	4.52	0.20	67.8
TJC_s-6	10	1.5	3.0	6.98	4.27	0.25	69.8
TJC_s-7	10	2.0	2.0	6.38	4.62	0.24	63.8
TJC_s-8	10	2.0	2.5	6.58	4.19	0.23	65.8
TJC_s-9	10	2.0	3.0	6.85	3.93	0.22	68.5
TJC_L-1	10	1.0	2.0	6.12	5.64	0.24	61.2
TJC_L-2	10	1.0	2.5	6.35	5.39	0.26	63.5
TJC_L-3	10	1.0	3.0	6.48	5.31	0.21	64.8
TJC_L-4	10	1.5	2.0	6.50	5.75	0.25	65.0
TJC_L-5	10	1.5	2.5	6.68	5.58	0.24	66.8
TJC_L-6	10	1.5	3.0	6.70	5.59	0.21	67.0
TJC_L-7	10	2.0	2.0	6.75	6.05	0.20	67.5
TJC_L-8	10	2.0	2.5	6.80	5.97	0.23	68.0
TJC_L-9	10	2.0	3.0	6.85	5.94	0.21	68.5

Table - 4 : It is apparent at optimum temperature i.e 40^0 and reaction time i.e 8 hrs which was standardized by other workers shows the conversion percentage using lipase from *J. gossipyfolia* shows higher yield i.e 68.5% in case of seed extract and 68.5% in leaf extract.

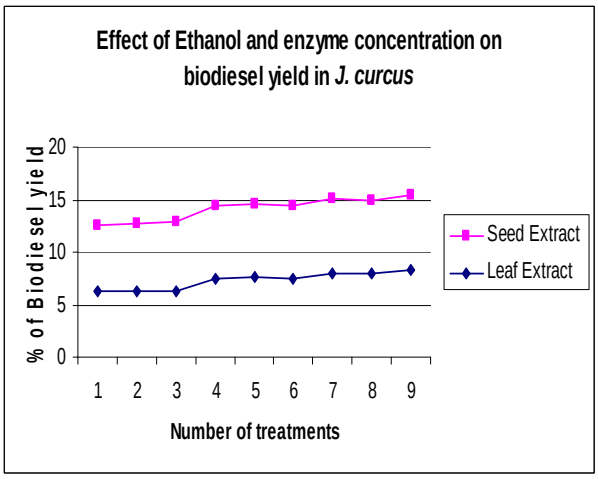

Figure-2: Effect of ethanol and enzyme concentration on biodiesel yield in *J. curcus*

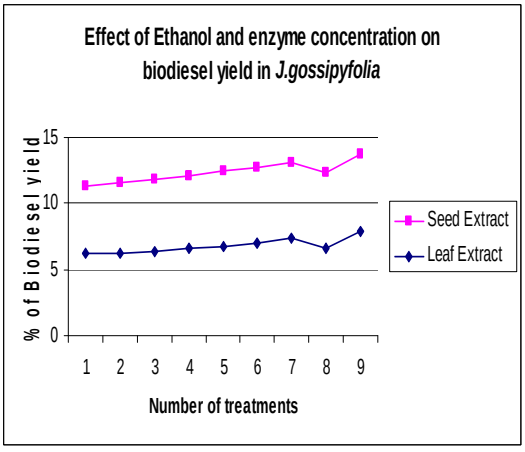

Figure-3: Effect of ethanol and enzyme concentration on biodiesel yield in *J. curcus*

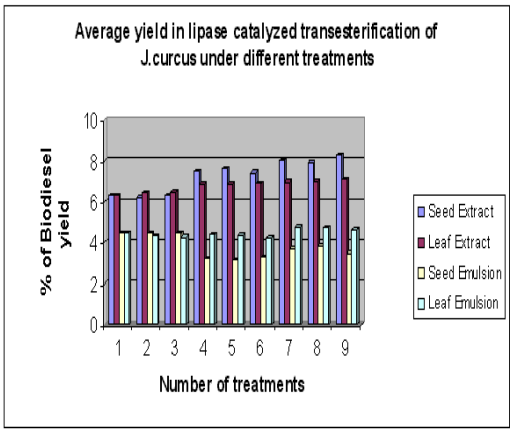

Figure-4: Average yield in lipase Catalyzed transesterification *J. curcus* under different treatments

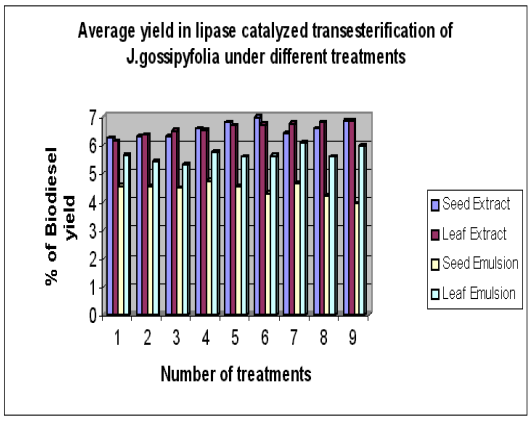

Figure-4: Average yield in lipase Catalyzed transesterification *J. goosiphfolia* under different treatments

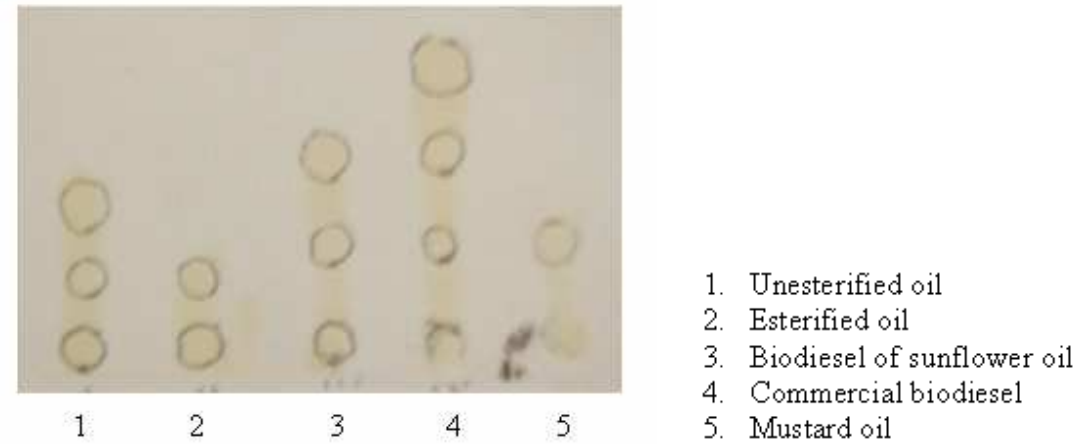

Figure-6: TLC observation of different oil samples

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Corresponding author:
R.K. Sahu
B.J.B. Autonomous College, Bhubaneswar, Orissa
Email: sahurajani@yahoo.co.in